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1 **Efficiency of biochar and compost (or composting) combined amendments for**
2 **reducing Cd, Cu, Zn and Pb bioavailability, mobility and ecological risk in**
3 **wetland soil**

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Abstract: Biochar and compost are two inexpensive and effective *in situ* remediation materials for heavy metal contaminated soils. The interaction between biochar and compost (or composting) calls further studies to maximize potential benefits of both. In this study, we examined short-time efficiency of compost (C), biochar (B), mixture of compost and biochar (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and biomass mixed before composting) for reducing bioavailability, mobility and ecological risk of Cd, Cu, Zn and Pb in wetland soil. Adding these amendment materials to the contaminated soil changed total organic carbon (TOC), water-extractable organic carbon (WEOC) and pH. All the materials decreased available Cd, Cu, Zn and Pb concentrations in soil (compost increased available Cu concentration) and Cd, Cu, Zn and Pb concentrations in pore water. As a whole, soil with Bced and BCing had the biggest decrease in these concentrations. These results indicated that all the materials reduced the bioavailability and mobility of heavy metals (compost improved bioavailability of Cu), and Bced and BCing had the greatest capacity for that. The materials improved soil microbial biomass and BCing created the biggest improvement, which suggested all the amendment materials reduced ecological risk of heavy metals and BCing had the greatest capacity for that.

Key words: Amendment; Heavy metal; Compost; Biochar; Soil microbial biomass

1. Introduction

Anthropogenic industrial and agricultural activities caused heavy metal (also is called potentially toxic metal) pollutants in extensive areas^{1, 2}. Heavy metals are difficult to degrade or remove in the environment. Pollution of heavy metal in soils may cause long-term risks to ecosystems and humans^{3, 4}. Accordingly, many techniques have been developed to remediate heavy metal polluted soils, including physical means, chemical means, incorporation of amendments, electrokinetic remediation, biological remediation and combined remediation technologies^{3, 5}. Modern remediation approaches increasingly focus on *in situ* environmentally friendly techniques, such as assisted natural attenuation and phytostabilisation often primed by the addition of soil amendments^{6, 7}. Compost (C), of the numerous amendment materials used for *in situ* stabilization of contaminants, has proven successful at binding heavy metals, rapid mobilization and vertical transport of trace metals^{6, 8-10}. Biochar (B), produced by pyrolysis of biomass under low oxygen conditions, has caught more and more attention as a soil amendment material^{8, 11}. Biochar has many favorable immobilization properties as a heavy metal modifier, such as a microporous structure, active functional groups, and high pH and cation exchange capacity (CEC)¹²⁻¹⁴. And it has been proved that biochar has a strong adsorptive power for heavy metals¹⁵⁻¹⁷.

As two of the important and inexpensive soil amendment materials, biochar and compost (or composting) also had influences on each other's properties. The

interaction of biochar and compost (or composting) has been reported in the recent years^{18, 19}. Addition of biochar could significantly influence the physic-chemical process and microbial community during the composting^{20, 21}, and also the composition and quality of the end product^{18, 22, 23}. Surface of biochar is modified during the composting process due to the biotic and abiotic oxidation, and sorption of compost-derived organic compounds²⁴⁻²⁶. The changes of these properties may influence the effectiveness of biochar and compost amendment for soil heavy metals.

Interaction of biochar and compost (or composting) could provide a method for improving the effectiveness of biochar and compost amendment. Biochar and compost mixed amendment material (B+C) had been studied widely in recent years. Beesley et al. found that B+C had higher efficiency for reducing water-extractable As and Cd in soil than that of biochar or compost, and higher efficiency for reducing Zn and Cd in soil pore water than that of biochar or compost⁸. Other study also found that B+C did not have higher efficiency for reducing mobility of heavy metal and As in a naturally contaminated mine soil than that of biochar²⁷. Borchard et al. reported composting increases the surface reactivity of biochars for Cu(II) sorption in water due to their uptake of compost-derived organic matter²⁵. The interaction between biochar and other organic amendment materials in soil should now be the focus of further study if we want to maximize the potential benefits of both⁶. However, little information is about the efficiency of composted biochar (Bced) or biochar-composting (BCing, biochar and biomass mixed before composting) on

contaminated soil *in situ* remediation.

In this study, we examined soil properties, concentrations of Cu, Zn, Pb and Cd in soil pore water and available Cu, Zn, Pb and Cd in soil after addition biochar, compost, B+C, Bcd and BCing to contaminated soil. Based on this work, the objectives of this study were: (1) to analyze the short-time efficiency of biochar and compost combined amendment materials for reducing heavy metals bioavailability and mobility; and (2) to examine the short-time efficiency of biochar and compost combined amendment materials for reducing heavy metals ecological risk, taking soil microbial biomass as an indicator.

2. Materials and methods

2.1. Soil and amendment materials

Soil (pH: 7.62; clay: 24.19 %, silt: 45.54 % and sand: 30.27 %) was sourced from beach of the Dongting Lake wetland. Dongting Lake, the second largest fresh lake in China, is located in the middle reach of Yangtze River region^{1,28}. The wetland is an important wintering habitat and pathway for East Asian migratory birds²⁹. Because of the mining wastewater, industrial wastewater and natural sources, the soil of Dongting Lake wetland was polluted by heavy metals^{28, 30-33}. The soil was collected from 10-20 cm soil depth on beach of the Dongting Lake wetland. The soil was air dried, sieved to a particle size of < 2 mm and biological debris was removed.

The whole procedure of the proposed method is shown in Fig. 1. Biochar was produced from corn cob at 450 °C using a slow pyrolysis method in a continuous flow

N₂ gas unit for a residence time of 1 h³⁴. All used biochar in this study was sieved to make sure its grain size was 0.125 mm~1.000 mm. Compost was produced from rice straw according to a previous study³⁵. B+C was made of the mixture of biochar and compost with the ratio of 1:1 (W/W). Bced was produced as follows: biochar was placed into mesh (0.125 mm) bags and then composted with rice straw, the bags filled with biochar was taken out after composting completed. BCing was produced as follows: biochar and rice straw were mixed (W/W: 1:1) and placed into the mesh bags and then composted with rice straw according above method. Chemical properties of these amendment materials are shown in Table 1.

2.2. Experimental design and procedure

The soil was thoroughly mixed with amendments in the following proportions, which constituted the treatments.

S: 500 g soil in each pot.

S + C: 500 g soil and 25 g compost in each pot.

S + B: 500 g soil and 25g biochar in each pot.

S + B + C: 500 g soil, 25 g B+C in each pot.

S + Bced: 500 g soil and 25 g Bced in each pot.

S + BCing: 500 g soil and 25 g BCing in each pot.

Finally, soil was placed into 1000 mL pots. Each treatment was implemented in triplicate.

One injection syringe of 15 mL was inserted into each pot, according to one

114 previous study ²⁷, to collect pore water. Deionized water was added to the soil of each
115 pot to achieve a fixed moisture content of 60% water filled pore space. These pots
116 were then placed in a controlled environment chamber with 28% relative humidity
117 and at 25 °C for 60 days. The water content of the soil in each pot was adjusted
118 weekly to maintain the water filled pore space of 60%. At day 7, 15, 30 and 60, pore
119 water was collected by replace the injection syringe. At day 60, the soil in each pot
120 was collected to analyses for soil properties, total metals, available heavy metals and
121 soil microbial biomass.

122 2.3. Analytical procedures for soil characterization

123 Amended soil pH (water: soil ratio of 1:2.5) was tested by a digital pH meter.
124 TOC of amended soil was examined by the loss-on-ignition method after ashing at
125 450 °C for 4 h ³⁶. Water-extractable organic carbon (WEOC) was obtained by
126 aggressive aqueous extraction using a 1:10 soil to deionized water suspension (2.5 g
127 soil: 25 g water), which was shaken for 3 h and centrifuged at 1408×g for 10 min,
128 then filtered ⁶. The filtered supernatant was determined using the Shimadzu TOC-V
129 CPH analyser (Shimadzu, Tokyo, Japan). Total metal content in amended soil was
130 determined by the AA700 atomic absorption spectrometry (PerkinElmer, USA) after
131 HNO₃-HF-HClO₄ digestion process ¹.

132 2.4. Measures of Cd, Cu, Zn and Pb bioavailability

133 Soil extraction method was evaluated using CaCl₂ solution as a surrogate
134 measures of metal bioavailability and ecotoxicity ³⁷. Extraction with 0.5 M CaCl₂

135 solution was completed according to one previous study^{38,39}. Extracted metal content
136 were measured using the above atomic absorption spectrometry.

137 2.5. Measures of Cd, Cu, Zn and Pb mobility

138 Metal content in pore water was determined as a surrogate measures of metal
139 mobility⁶. Cd, Cu, Zn and Pb contents in pore water were measured by the above
140 atomic absorption spectrometry.

141 2.6. Ecological risk

142 Soil microbial biomass was used to assess the ecological risk of metals in soil⁴⁰.
143 As a representative for soil microbial biomass, microbial biomass carbon (MBC) was
144 determined by the fumigation-extraction method⁴¹. K₂SO₄-extracted C content was
145 examined with the Shimadzu TOC-V CPH analyser (Shimadzu, Tokyo, Japan). The
146 MBC was calculated as the difference in extractable C between fumigated and
147 un-fumigated samples using a conversion factor of 0.37⁴².

148 2.7. Statistical analyses

149 One way analysis of variance (ANOVA), using Tukey test, was used to
150 determine differences between each soil treatments. Correlation analysis was
151 completed to determine the relationships between MBC and other examined
152 parameters. All these analyses were conducted using SPSS (version 11.5).

153 3. Results

154 3.1. Effects of amendments on soil characteristics

155 TOC, WEOC and pH of each treatment are shown in Fig. 2. All amendment

156 materials increased the TOC of soil. Compost had the smallest increase and biochar
157 had the biggest increase. However, biochar had no significant effect on the WEOC
158 and others caused obvious increase in that. The increase caused by B+C was the
159 smallest increase and the increase caused by BCing was the biggest increase. The
160 contrasting effects of amendments on the pH, compared to the TOC and WEOC, were
161 B and B+C had no obvious change ($S+B > S > S+B+C$) on pH and others had a
162 decrease.

163 Total heavy metals are shown in Fig. 3. And the total concentration of each metal
164 of soil without amendment was almost the biggest one. Most of amendments had no
165 significant effect on the total heavy metals. In stark contrast, these amendments had
166 significant impact on the available heavy metals (Fig. 3). The available concentration
167 of each element was far lower than its total concentration. Compost increased the
168 concentration of available Cu and other amendments effectively reduced the
169 concentration. All the amendments effectively decreased the concentration of
170 available Zn, Cd and Pb. Bced and BCing had the highest efficiency in decreasing the
171 concentration of available heavy metal. The efficiency of BCing was slightly above
172 that of Bced. Among the different elements, available Zn had the biggest decrease and
173 available Cd had the smallest decrease.

174 3.2. Effects of amendments on heavy metal concentration in pore water

175 All the amendments effectively decreased the concentration of heavy metals in
176 pore water (Fig. 4). In 7th day, compost increased Cu concentration in pore water.

Other amendments reduced Cu concentration in pore water and BCing had the greatest magnitude of effect. The Cu concentration in pore water with addition of compost or BCing was decreased following time. The hierarchy in the effectiveness of amendments for decreasing Zn in pore water, comparing to the no amendment soil, was as follows: compost < biochar < B+C < Bced < BCing. The reduction in average of Cd concentration with addition of compost, biochar, B+C, Bced and BCing was 89.97%, 97.35%, 92.42%, 98.36% and 98.55%, respectively. Compost and biochar had similar efficiency in reducing Pb concentration in pore water, Bced and BCing had slightly higher efficiency.

3.3. Effects of amendments on soil microbial biomass

There was significant difference in soil microbial biomass between each treatment (Fig. 5). All the amendments had improved MBC of soil. MBC of soil with BCing had the biggest increase and that of soil with biochar had the smallest increase. The results of Pearson's correlation analysis are shown in Table 2. WEOC was strongly correlated ($P = 0.036$) with MBC. TOC ($P = 0.137$) and pH ($P = 0.153$) were not strongly correlated with MBC. There were significant negative correlations between MBC and available Zn ($P = 0.045$), Cd ($P = 0.021$) and Pb ($P = 0.048$), indistinctive negative correlation ($P = 0.348$) between MBC and available Cu. The order of correlation coefficients absolute value was: Available Cd > WEOC > Available Zn > Available Pb > TOC > pH > Available Cu.

4. Discussion

198 All the amendment materials, as organic amendment materials, improved TOC of
199 the treated soil. Biochar increased more TOC because of its higher TOC, and compost
200 increased less TOC because of its lower TOC. However, WEOC of soil with biochar
201 increased less than that of others amendments, because that carbon pool of biochar is
202 relatively stable and insoluble ^{6, 27}. Other studies ^{43, 44} also did not find an obvious
203 change in concentration of WEOC caused by biochar. Effects of combined
204 amendment (B+C, Bced and BCing) on soil TOC and WEOC were the results of
205 combined impact of compost and biochar. Compost addition, whether alone or
206 combined with biochar (B+C, Bced and BCing) resulted in reduction of soil pH. This
207 is because of humic acids isolated from organic materials of compost. Other study ²⁴
208 also found Bced reduced pH of neutral soil while biochar did not change pH. Total
209 heavy metals of soil with all amendment materials almost were lower than that of soil
210 without amendment materials. This could be attributed, in part to the dilution of the
211 original contaminated substrate by amendment materials applied ⁶.

212 Comparison of available Cd, Cu, Zn and Pb, TOC and WEOC between each
213 treatment showed that the organic amendment materials can effectively reduce
214 bioavailability of heavy metal (compost increase bioavailability of Cu) and improved
215 TOC and WEOC. The reason for this was the phenomenon that amended soil with
216 highly organic materials can generate large concentrations of WEOC to which free
217 ions can complex with organic ligands ^{27, 45, 46}. Besides, heavy metals exchange with
218 Ca^{2+} , Mg^{2+} and other cation associated ^{47, 48}. All these increase the concentrations of

219 carbonate fraction, Fe and Mn fraction, organic matter bound fraction and residual
220 fraction of heavy metal, and reduced available fraction. Bced and BCing have the
221 greatest ability for improving WEOC of soil and also contain function of biochar.
222 Therefore, Bced and BCing had the greatest efficiency for reducing bioavailability of
223 heavy metal. Other study also found compost increased the concentration of available
224 Cu and it because Cu was slightly mobilized by the humic acids¹⁰.

225 Concentration of heavy metal in pore water suggested that all the amendment
226 materials could stabilize heavy metal and reduced heavy metal mobility. Compost
227 could stabilize heavy metal because heavy metals in compost amended soil was
228 inextricably linked to organic carbon turnover⁴⁹. Stabilization of heavy metals in soils
229 with application of biochar could involve a number of possible mechanisms that could
230 include (1) heavy metal exchange with Ca^{2+} , Mg^{2+} and other cations associated
231 biochar, attributing to co-precipitation inner-sphere complexation with complexed
232 humic matter and mineral oxides of biochar; (2) the surface complexation of heavy
233 metals with different functional groups, and inner-sphere complexation with the free
234 hydroxyl of mineral oxides and other surface precipitation; and (3) the physical
235 adsorption and surface precipitation^{47, 48}. According to this study, we could found that
236 Bced and BCing had the greatest efficiency of reducing heavy metal mobility. This
237 presumably is attributed to the phenomenon that composting process strongly
238 increased biochar's CEC and O-content⁵⁰, which was related to sorption of heavy
239 metal. The increase in CEC and O-content may be caused by biologically mediated

240 oxidation of biochar surfaces ⁵¹⁻⁵³ and/or strong sorption of organic matter during
241 composting ^{50, 54}.

242 Contaminants may affect the microbial processes in soil, thereby affect the
243 nutrients cycling and the capacity to perform key ecological functions, such as
244 mineralization of organic compounds and synthesis of organic matter ⁴⁰. Soil
245 microbial biomass appears to be sensitive and responsive to changing environmental
246 conditions ^{55, 56} and can be used as an indicator of ecological risk assessment of soil
247 contamination ⁴⁰. In this study, we found that MBC was strongly negatively correlated
248 with available Zn, Cd and Pb. The difference of MBC in this study means that BCing
249 had the greatest efficiency for reducing ecological risk, and biochar had the weakest
250 efficiency for that. Bced and BCing significantly changed soil microbial biomass and
251 also affected soil microbial community structure, which played an important role in
252 many ecological processes. How Bced and BCing affect soil microbial community
253 structure needs further studies. Tripathy et al. found that MBC/OC was significantly
254 and negatively correlated with water-soluble and exchangeable metals (Zn, Cu, Pb, Cr
255 and Ni) and claimed that labile metal forms such as water-soluble and exchangeable
256 fractions are the most important factors regulating microbial biomass in soil ⁵⁷.
257 Besides, other studies found MBC was strongly correlated with TOC because organic
258 matter was a substrate for microbial growth ^{55, 56, 58, 59}. However, in this study we
259 found that MBC was strongly correlated with WEOC and was not strongly correlated
260 with TOC. This is attributed to the fact that carbon pool of biochar was relatively

261 insoluble and stable^{6,27}, which could not be digested by soil microbes.

262 **5. Conclusions**

263 Adding compost (C), biochar (B), mixture of compost and biochar (B+C),
264 composted biochar (Bced) and biochar-composting (BCing, biochar and biomass
265 mixed before composting) to contaminated soil changed soil physic-chemical
266 properties, such as TOC, WEOC and pH. The changes of available Cd, Cu, Zn and Pb
267 suggested that all the amendments reduced bioavailability of heavy metal (compost
268 improved bioavailability of Cu), and Bced and BCing had the greatest capacity for
269 that. The difference of Cd, Cu, Zn and Pb concentration in pore water between each
270 treatment showed that all the amendments reduced mobility of heavy metal, and Bced
271 and BCing had the greatest capacity for that. Comparison of MBC between each
272 treatment declared amendments reduced ecological risk of heavy metal, and BCing
273 had the greatest capacity for that. Influences of Bced and BCing on soil microbial
274 community structure need further studies.

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387 **Figure captions**

388 **Fig.1.** The whole procedure of the proposed method.

389

390 **Fig.2.** TOC, water-extractable organic carbon (WEOC) and pH of soil with addition
391 of nothing (S), compost (C), biochar (B), mixture of biochar and compost (B+C),
392 composted biochar (Bced) and biochar-composting (BCing, biochar and biomass
393 mixed before composting). Error bars represent standard deviation ($n = 3$). Different
394 letters indicate significant difference ($p < 0.05$) between each treatment.

395

396 **Fig.3.** Concentration of total heavy metal and available heavy metal of soil with
397 addition of nothing (S), compost (C), biochar (B), mixture of biochar and compost
398 (B+C), composted biochar (Bced) and biochar-composting (BCing, biochar and
399 biomass mixed before composting). Error bars represent standard deviation ($n = 3$).
400 Different letters indicate significant difference ($p < 0.05$) between each treatment.
401 Transverse lines were the local background values of total metals (According to the
402 Environmental Quality Report (2011) of Hunan Province).

403

404 **Fig.4.** Heavy metal concentration in pore water from soil with addition of nothing (S),
405 compost (C), biochar (B), mixture of biochar and compost (B+C), composted biochar
406 (Bced) and biochar-composting (BCing, biochar and biomass mixed before
407 composting). Error bars represent standard deviation ($n = 3$). Different letters indicate
408 significant difference ($p < 0.05$) between each treatment in the same time.

409

410 **Fig.5.** Microbial biomass carbon (MBC) of soil with addition of nothing (S), compost
411 (C), biochar (B), mixture of biochar and compost (B+C), composted biochar (Bced)
412 and biochar-composting (BCing, biochar and biomass mixed before composting).
413 Error bars represent standard deviation (n = 3). Different letters indicate significant
414 difference ($p<0.05$) between each treatment.

415 **Table 1.** Chemical properties (means $\pm$ SD, n=3) of compost (C), biochar (B),
 416 composted biochar (Bced) and biochar-composting (BCing, biochar and biomass
 417 mixed before composting).

Property	C	B	Bced	BCing
pH	6.72 $\pm$ 0.02	9.98 $\pm$ 0.01	7.13 $\pm$ 0.04	7.04 $\pm$ 0.01
TOC ^a (%)	30.25 $\pm$ 1.02	55.97 $\pm$ 2.41	57.16 $\pm$ 3.63	51.80 $\pm$ 0.73
WEOC ^b (g/kg)	28.77 $\pm$ 3.56	1.84 $\pm$ 0.23	6.91 $\pm$ 0.20	31.27 $\pm$ 1.34
CEC ^c (cmol _c /kg)	85.22 $\pm$ 3.85	60.93 $\pm$ 2.71	118.57 $\pm$ 2.09	131.06 $\pm$ 3.54
O-content (%)	15.36 $\pm$ 0.23	9.64 $\pm$ 0.19	12.59 $\pm$ 0.17	13.98 $\pm$ 0.19

418 ^a total organic carbon.

419 ^b water-extractable organic carbon.

420 ^c Cation exchange capacity.

421 **Table 2.** Pearson’s correlation coefficients between MBC and other soil parameters.

Parameters	Correlations coefficient	P-value
pH	-0.661	0.153
TOC	0.681	0.137
WEOC	0.840	0.036
Available Cu	-0.469	0.348
Available Zn	-0.821	0.045
Available Cd	-0.879	0.021
Available Pb	-0.815	0.048

422

Fig.1

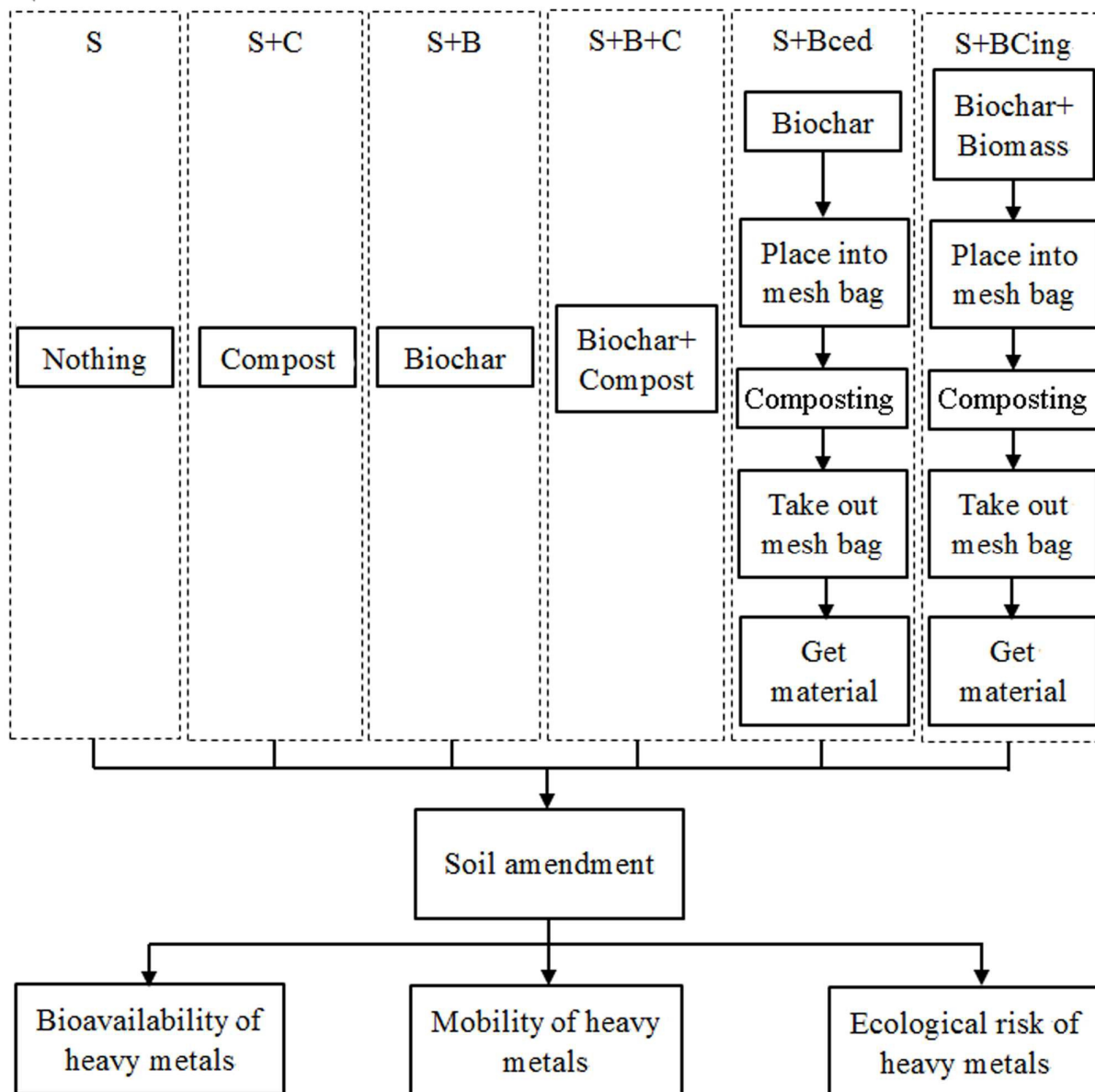


Fig.2

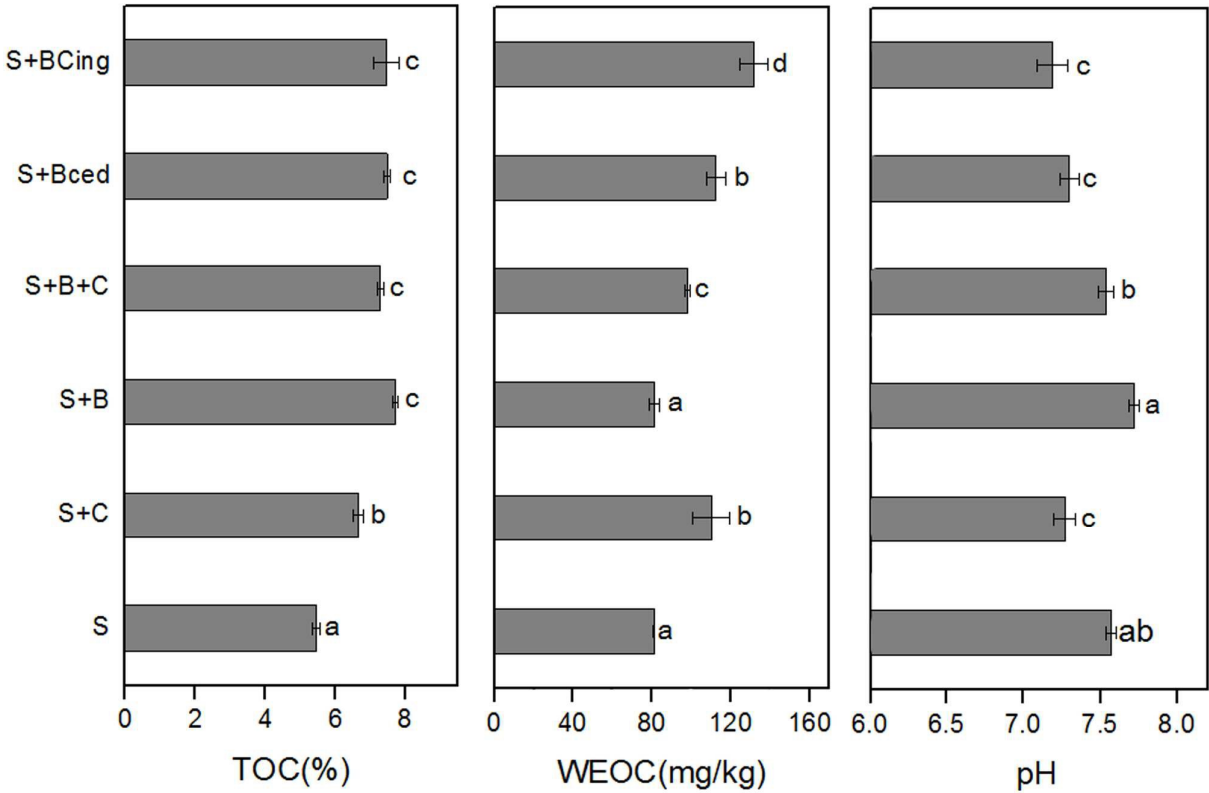


Fig.3

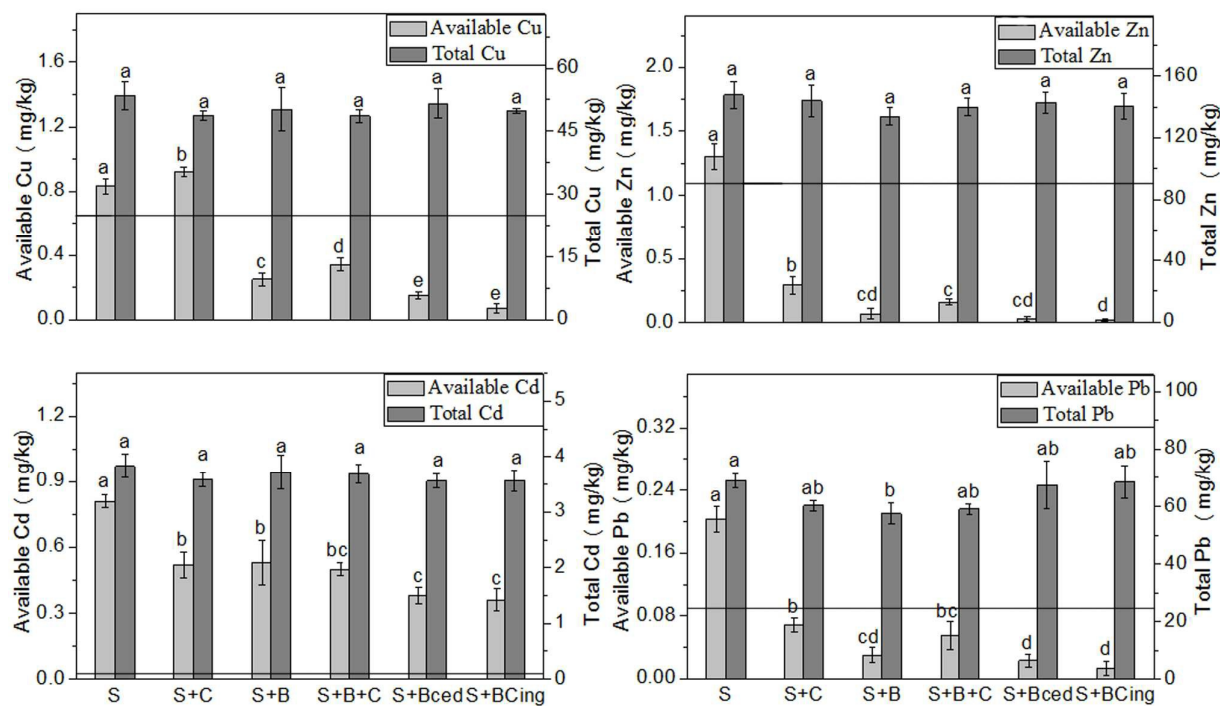


Fig.4

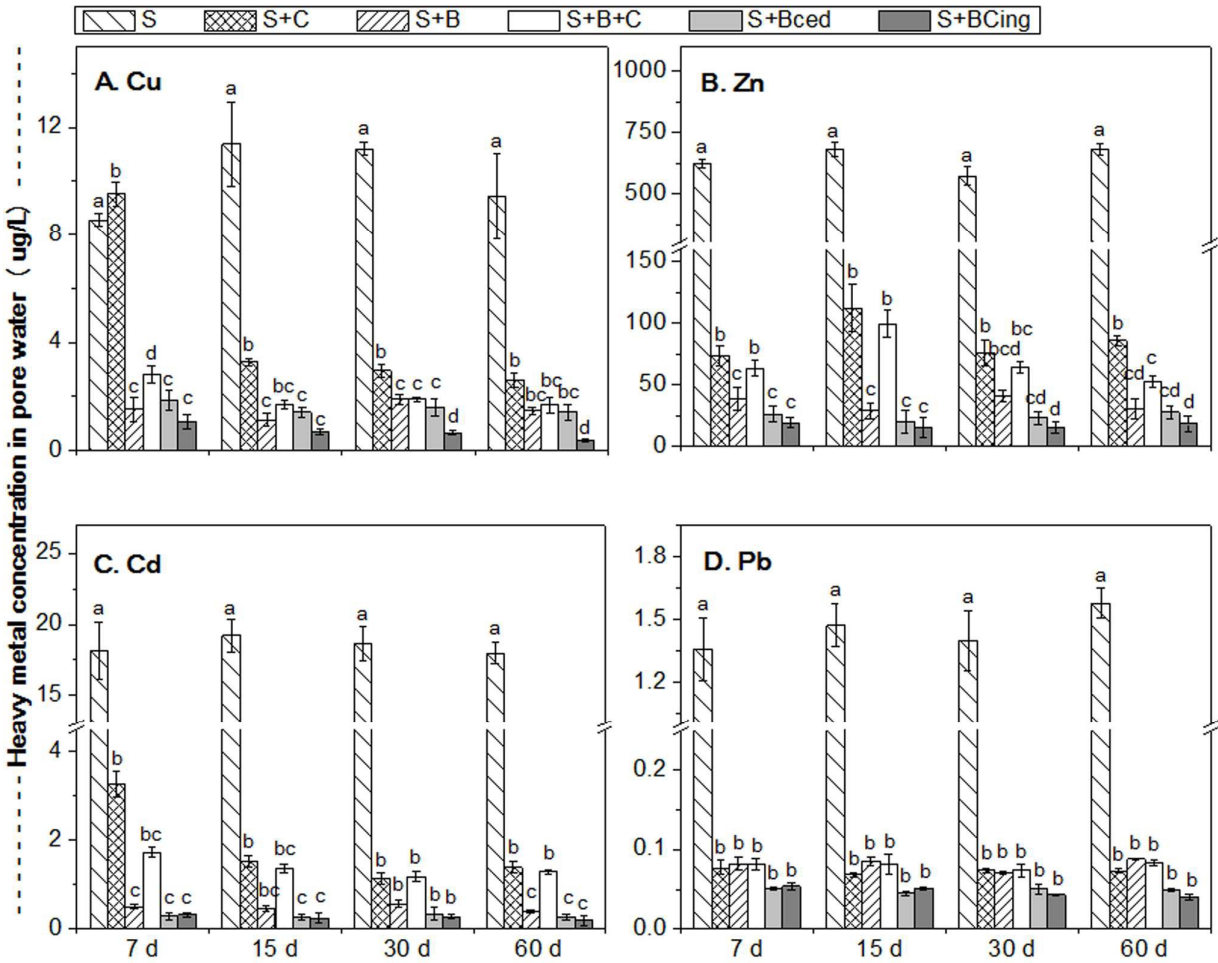


Fig.5

